LIPOSOMAL DELIVERY VEHICLES OF PACLITAXEL

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Abstract - **Paclitaxel stabilizes microtubules while inhibits mitotic spindle formation. It has been found to be effective in treating several solid cancers. The aim of this study was to incooperate paclitaxel in conventional and PEGylated liposomes, and to evaluate the antiproliferative effects of different formulations on MCF-7 and ZR75-1 cell lines. MTT assay was used to determine the growth inhibition of the cell line by paclitaxel. A significant dose-dependent inhibition of proliferation was found after the cells were exposured to certain liposomal paclitaxel preparations, suggesting the possible use of liposomes as effective palitaxel delivery devices.**

Keywords - **paclitaxel; liposome; breast cancer; cell; MTT.**

Ⅰ. INTREODUCTION

Breast carcinoma cells grow in situ as solid tumor masses. The MCF-7 and ZR75-1 cell line, derived from breast carcinomas, grow in tissue culture in monolayers with an epithelial sheet-like morphology. Recently, paclitaxel has been introduced as a novel anticancer agent showing activity against a broad range of human tumors especially drug-resistant ovarian and breast carcinomas. The main molecular target of paclitaxel is the equilibrium between microtubules and tubulin dimers, which form the basic units of microtubules. The ability of paclitaxel to stabilize polymerized tubulin into microtubule bundles results in an efficient inhibition of replication, locking the cells in the late G_2 or M phase of the cell cycle.

Other effects of paclitaxel have also been suggested, such as to induce internucleosomal DNA fragmentation and apoptosis, to increase TNF-2 (tumour necrosis factor-2) mediated cell cytolysis, and to interfere with tumour cell invasion and metastasis. Furthermore, paclitaxel has been shown to play a role in modulating the interaction of growth factors with their corresponding receptors and the resulted intracellular signaling pathways.

The commercial formulation of paclitaxel consists of a micellar solution of the drug in Cremophor EL (polyoxyethylated castor oil) containing 50% ethanol. Since Cremophor EL has been observed to cause severe hypersensitive reactions in animals and man, alternative formulations, such as micelles, emulsions and nanosuspensions have been investigated. Adopting other formulations, however, may change the drug interaction made with cancer cell, therefore, their therapeutic efficiencies.

The aim of the present investigation is to analyze the antiproliferative effects of different paclitaxel formulation on MCF-7 and ZR75-1 cells., and to compare the efficiencies and the time course of their effects.

Ⅱ. MATERIAL AND METHODS

1. Cells culture

MCF-7 and ZR75-1 breast cancer cells were obtained from the American Type Culture

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Collection (ATCC) and they were routinely cultured in modified Eagle's Medium (MEM, Gibco) containing 10% heat inactivated fetal bovin serum (FBS), 1% L-glutamine, 50 μ /ml penicilin and 50 μ /ml streptomycin, using a standard protocol. Cells were maintained at 37℃ in a humidified atmosphere of 95% air and 5% CO2. Cells were in the logarithmic phase of growth at the time of the drug sensitivity assays.

2. Preparation of paclitaxel-containing liposome

The lipids were dissolved in chloroform, paclitaxel in chloroform, mixed and dried under vacuum. Buffers were then added to hydrate the file and form liposomes. To obtain small and homogeneous vesicles, the liposomes suspension was extrudes 10 cycles each through polycarbonate filters with 0.2 and 0.1 μ m pores.

To assay for incooperation efficiency the liposome were spun down into a pellet and separated from the supernatant. The amounts of paclitaxel in both fractions were determined by HPLC (Agilent1100.USA). The column was eluted with acetonitrile: water (66:34). Detection was by UV absorption measurement at 227nm (flow rate 1ml/min).

3. Determination of cytotoxicity

Drug sensitivity was determined using a standard colorimetric MTT (3-4,5-dimethylthiazol-2-yl-2, 5-diphenyl-tetrazolium bromide) assay. Briefly, cells were plated out at a density of 10^4 cells/100 μ l/well in 96-well microtitre plates and allowed an overnight period for attachment. Then the medium was removed and fresh medium, along with various concentrations of paclitaxel, control cultures containing no paclitaxel were set up in conditions otherwise identical. Triplicate plates of each of the treated and control cultures in three separate independent experiments were incubated for 5 days at 37℃ in a humidified 5% CO_2 incubator. Following treatment, cells were fed with MTT (10 μ l/well, 5mg per ml in PBS) and incubation was prolonged for 3h at 37℃. After removing the supernatants, The MTT-formazan crystals were dissolved in DMSO (100 μ l/well) and the absorbance was measures at 570nm in a multi-well plated reader (Model Anthos Labtec 2010.7 reader). The percent viability of each well was calculated from the following:

$$\text{Percent viability} = \frac{A-B}{C-B} x100\% \qquad (1)$$

A- absorbance of test;
B- absorbance of blank;
C- absorbance of control.

The data presented are the mean±standard derivation from three replicated wells per microwell plate and three replicate microwell per cell line. Data from the MTT assays were analyzed by means of Student's t-test. AP-value less than 0.05 was considered to be significant.

The 50% inhibitory drug concentration (IC50 value) was statistically determined using SSPS (probit-analysis).

III. RESULTS

Paclitaxel liposome formulations were prepared by extruding of multilamellar liposomes using different phospholipid mixtures. Hydration of the drug-lipid film, followed by 10 cycles each of extrusion through 0.2 μ m, 0.1 μ m polycarbonate filters, was found to be a feasible preparation method for homogeneous small unilamellar vesicles.

Different liposomal formulations were used to entrap paclitaxel, varying liposome properties such as membrane fluidity and surface modification. The formulations are to substantial entrapment of paclitaxel in liposomal formulations were generally achievable despite the lipid composition both chemically and physically stable under physiological conditions for at least 1 month.

When cell were exposed to paclitaxel, MTT utilization decreased in a dose-dependent manner. The differences in MTT utilization between control and treated MCF-7 and ZR75-1 cells were determined to calculate cell viability. Beginning with 0.01 μ m paclitaxel. There is about 70% cell viability. Whereas at >0.1 μ M paclitaxel, cells died resulting a significant decrease in cell numbers. At 0.1μM paclitaxel, about 62% cells left. When paclitaxel is 1.0μM, the cell viability is only 18%. Such profile changed significantly with various liposome formulations.

Ⅳ. DISCUSSION

Liposomal formulations of paclitaxel have been suggested to have many advantages over current commercial formulation containing Cremophor EL. Varies lipid compositions were tested for better loading efficiency and storage stability. Further more, for in vivo stability and tumor-targeting effect, PEGylated lipids may also be included. Certainly, such improvements in formulation will definitely change various pharmacokinetic aspects of the drug. There have been several extensive studies about it. But at the same time, we think it is also important to look into the change of their interaction mode with cells.

Here we established a model system using breast cancer cultured cells. The interactions between free paclitaxel molecule and the cells, and between paclitaxel containing liposomes and the cells are considerably different. In this report, we focus on the antiproliferative efficiencies of the various liposome formulations and the time courses of their effects.

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